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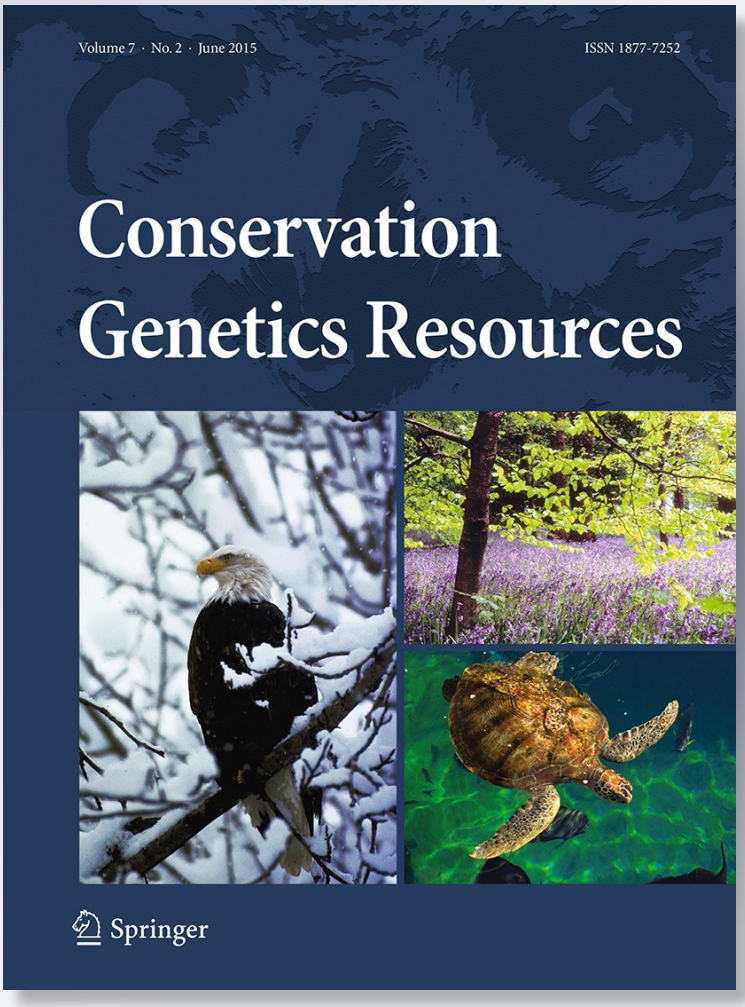

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MICROSATELLITE LETTERS

Development and characterization of polymorphic microsatellite markers for the crested caracara, *Caracara cheriway*

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Abstract We isolated novel microsatellites from the crested caracara (*Caracara cheriway*) with a shotgun pyrosequencing approach. We tested 80 loci for polymorphism among 20 individuals from the threatened Florida population. Fourteen loci were polymorphic. The mean number of alleles was 2.21 (range 2–3) and the mean observed heterozygosity was 0.41 (range 0.15–0.65). None of the 14 polymorphic loci exhibited significant linkage disequilibrium nor did they deviate significantly from Hardy–Weinberg expectations. We also report 16 monomorphic loci.

Keywords Microsatellite · Falconidae · Florida · 454 Pyrosequencing

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The crested caracara (*Caracara cheriway*) is a medium-sized raptor found commonly throughout Mexico, Central America, and northern regions of South America; it also occurs sparsely in the southern United States. There are currently stable populations in Texas while a small population in Florida is listed as threatened (Morrison and Dwyer 2012). The decline of the Florida population is primarily attributed to habitat loss due to rapid urbanization of agricultural and pasture lands (Morrison and Humphrey 2001). To date, no genetic analyses have been conducted on any population of caracara. To supplement current efforts to restore the Florida population and measure genetic diversity within the species, we have developed microsatellite primers from *Caracara cheriway* genomic data.

We used 2 μg of genomic DNA to prepare a library for sequencing at the University of Arizona Genetics Core. We used ¼ plate on the 454 GS FLX platform (Roche Applied Science) to sequence our library. This run generated 371,387 raw individual reads for a total of 137.8 Mbp. The reads were trimmed to eliminate the first 4 bp corresponding to the library tag. Also, the 3′ end of each sequence was trimmed until the Q20 criterion was met over a 10 bp window. The resulting mean read length was 372 bases (SD = 160). We used the program QDD (Meglécz et al. 2010) to search within quality-filtered reads to identify and design primers for microsatellite loci containing a minimum of six perfect repeats for dinucleotides and five perfect repeats for larger motifs. From 3,801 dinucleotide, 1,424 trinucleotide, 392 tetranucleotide, 195 pentanucleotide, and 49 hexanucleotide repeat loci found, QDD designed primers for 1,629 unduplicated loci. From these unduplicated loci, we selected tetranucleotide repeat loci with greater than six repeats (41 loci) and dinucleotide repeat loci with >11 repeats (39 loci) for primer synthesis.

Table 1 Characterization of 30 microsatellite loci isolated from *Caracara cheriway* and tested in 20 individuals from Florida

Locus	GenBank accession no.	Repeat motif	Primer sequences	Size range (bp)	No. alleles	H_o	H_e
CRCA1	KJ169554	AAAT	F: GCAATACATCGGAGACAGGC	289–297	3	0.60	0.66
			R: TGTTGGTCTCAGGGCAGAGT				
CRCA2	KJ169555	AAAT	F: GCGTGATAGGTGAAATGCAA	146–150	2	0.15	0.14
			R: TCAGGCTTGGAATCCTGTTT				
CRCA3	KJ169556	AGGT	F: TCTGGAAATCCGTATCTACCCA	136–144	3	0.50	0.49
			R: AACTGAAATTTGTCTTGCAGAGG				
CRCA4	KJ169557	ACGG	F: AGTCTGAACGCTGAAGTGTAGG	117–121	2	0.45	0.47
			R: AGCCTTTGCAATGAAGCAAT				
CRCA5	KJ169558	AC	F: ACCTCTGGCAAGAGTGCTCA	196–202	2	0.20	0.18
			R: GGTGGGACTGTGCAATGTAA				
CRCA6	KJ169559	AC	F: GCAGCAATGTACTCTCTTGCAG	164–174	2	0.25	0.35
			R: TGCAGAGAGATCGGGATGTT				
CRCA7	KJ169560	AC	F: GTCGCTGTTAGTACCGTGGC	130–140	3	0.45	0.53
			R: TGAACTGGTCTATGTGCGCC				
CRCA8	KJ169561	AC	F: CAGAGGTCTGGGTTTCTGTGA	301–303	2	0.60	0.5
			R: CCACTCCTGGGAACAGTTTG				
CRCA9	KJ169562	AC	F: TCCATAAGCCTCACCACCAT	123–129	2	0.45	0.49
			R: GCTTTCAGCTGCCAGTCAGT				
CRCA10	KJ169563	AC	F: AATCTAGCTCCCAGCCAAGC	163	1	–	–
			R: CATGTCTTCCTAGAGTTGCCTTT				
CRCA11	KJ169564	AC	F: TTGGTTCCTCGTTTACTACACC	147–151	2	0.45	0.35
			R: TCAAGACTTGCCACCCTCAT				
CRCA12	KJ169565	AC	F: TGATCTCTGGTGTGCTTGTAGG	108–114	2	0.45	0.49
			R: AAGAATTAGAAGTGGTCGTCTTCG				
CRCA13	KJ169566	AT	F: AGGGTCTGGATAGATAAGGCTC	263–265	2	0.65	0.50
			R: AGAACAGCATTTCTCCTGCG				
CRCA14	KJ169567	AC	F: GGAATGTGTCAGAACAGTTTGC	312–316	2	0.20	0.32
			R: GCAGCACCCTGTAAGAATGG				
CRCA15	KJ728767	AC	F: TGCAAGTGGTGGAAGGAAGT	207–209	2	0.30	0.26
			R: GGGCAGCAGAACAGCAGTAT				
CRCA16	KJ728768	AAAC	F: TGAAAGAACAGTAGCATCCCAG	285	1	–	–
			R: TGAACAGTTTAACATCAAATCCTGA				
CRCA17	KJ728769	AAAC	F: TGATGTTGACCTTCCTGCAC	176	1	–	–
			R: CATCAGAAATCAGGGATGGG				
CRCA18	KJ728782	AAAT	F: TCCGTGATATTGCACGCTTA	215	1	–	–
			R: CCGTCAGCTTCCGTAGTTTG				
CRCA19	KJ728770	ACAG	F: CTTCCACAAGATGGAGCCTG	182	1	–	–
			R: GCCATTCTGCATCGCTCTC				
CRCA20	KJ728771	AAAT	F: GAGGCCTTTCCAAGGTGAAT	190	1	–	–
			R: TTTCTCCTTGCTGGCAACTT				
CRCA21	KJ728772	AAAT	F: AGGATGGACAGCCTTCATCA	175	1	–	–
			R: CCATTTCAGGGAGTGACAGG				
CRCA22	KJ728773	ACCT	F: ATAGCTCAGCAGGTCCCACA	306	1	–	–
			R: CGCTACCATTTCCAGGCTTA				
CRCA23	KJ728774	AC	F: GCCATGTGTAGCAAAGGACC	144	1	–	–
			R: GCGTGTGTGCTTGCATGTAT				
CRCA24	KJ728775	AC	F: TATGCAATGTGCAGGCATGT	115	1	–	–
			R: ACCCGGTACTCAGCAATCTG				

Table 1 continued

Locus	GenBank accession no.	Repeat motif	Primer sequences	Size range (bp)	No. alleles	H_o	H_e
CRCA25	KJ728776	AC	F: TTACTTCAGTCATGTCCTTCTCTTT	169	1	–	–
			R: CGGGAATCCTACAGGTAGCC				
CRCA26	KJ728777	AG	F: ACAACTGCCTGAATTCCACA	185	1	–	–
			R: TAGATGCTCCAGCCTGCATT				
CRCA27	KJ728778	AC	F: TTTCTACATGCACGAGACGG	119	1	–	–
			R: TGGCTACTAGCACTGCTGCTT				
CRCA28	KJ728779	AG	F: TGCTCACTTGCCCAAATGTT	205	1	–	–
			R: CTGGAGAATCTGCCGTATGC				
CRCA29	KJ728780	AG	F: TTTGTCCACAACACTGGTGC	321	1	–	–
			R: ATGGCTGGCTCATCTTTCTT				
CRCA30	KJ728781	AC	F: GCTGGACCACACCAATAAGC	298	1	–	–
			R: TGCGTTAATTATTGCCCTTG				

We added the M13 primer sequence at the 5′ end of all forward primers for economical fluorescent labeling and a "pig-tail" sequence (GTGTCTT) to the 5′ end of all reverse primers to reduce ambiguity in fragment size due to incomplete adenylation. We conducted PCRs in 15 µl volumes with 10–20 ng genomic DNA, 1X PCR buffer, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 0.5 U *Taq* DNA Polymerase (New England Biolabs), 0.2 µM unlabeled M13-tailed forward primer, 1 µM 6-FAM-labeled M13 primer, and 1 µM reverse primer. We used a touchdown PCR protocol consisting of 94 °C for 5 min, 12 cycles of 95 °C for 30 s, 67–55 °C for 20 s (1 °C decrease each cycle), 72 °C for 20 s, followed by 33 cycles at 95 °C for 30 s, 55 °C for 20 s, 72 °C for 20 s, and a final extension of 72 °C for 30 min. We used an ABI3730 DNA analyzer (Applied Biosystems) to genotype PCR products and scored alleles in GENOTYPER version 3.7 (Applied Biosystems). To estimate deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) we used GENEPOP version 4.2 (Rousset 2008) and corrected for multiple comparisons using a sequential Bonferroni test ($\alpha = 0.05$). Finally, we used MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) to test for null alleles and allele drop-out.

Thirty loci successfully amplified, of which, 14 (four tetranucleotide and ten dinucleotide motifs) were polymorphic within a set of 20 individuals from the Florida caracara population (Table 1). The mean number of alleles per locus was 2.21 (range 2–3) and the mean observed heterozygosity was 0.41 (range 0.15–0.65) (Table 1). We detected no significant LD among loci pairs, deviation from HWE, or evidence for null alleles and allele drop out (adjusted $p > 0.05$). Sixteen loci (seven tetranucleotide and nine dinucleotide motifs) amplified well but were monomorphic within the same set of 20 individuals. We have reported the monomorphic loci here (Table 1) as they may be found to be polymorphic in other populations of crested caracara. Sequences for all 30 loci are provided (Online Resource 1).

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